

69. (new) The method of claim 68 wherein said N-terminal portion of a mature plastid protein comprises between 15 and 25 amino acids of the N-terminal portion of the mature plastid protein.

70. (new) The method of claim 25 wherein said transit peptide comprises a plant plastid transit peptide, an N-terminal portion of a mature plastid protein linked by its N-terminus to the C-terminus of said plastid transit peptide, and a second plastid transit peptide linked by its N-terminus to the C-terminus of said N-terminal portion of a mature plastid protein.

71. (new) The method of claim 70 wherein said transit peptide is an optimized transit peptide (OTP) comprised of the sunflower RuBisCO ssu transit peptide fused to a peptide made of the twenty-two N-terminal amino acids of the mature maize RuBisCO ssu, which is in turn fused to the to the maize RuBisCO ssu transit peptide.

REMARKS

Claims 1-59 are pending in the application. Claims 7, 10, 11, 14-16, 21, 22 and 31-59 have been withdrawn from consideration. Claims 2-6, 9, 12, 13, 17-20 and 23-25 have been amended. Claims 1, 8, 14, and 27-30 have been canceled without prejudice and replaced with new claims 60-64 and 66-71 and follows:

Canceled Claim	New Claim
1	60
8	61, 62
14	63, 64
27	66, 67, 68
28	69
29	70
30	71

Claim 14, which was withdrawn from consideration, has been replaced with new claims 63 and 64. Applicants request that claim 14, now rewritten as new claims 63 and 64, be considered at the present time. Claim 14 related to the use of mitochondrial transit peptides in the method of the invention to obtain overexpression of serine acetyltransferase (SAT) in the mitochondria, not to a mitochondrial SAT *per se*, and properly belongs in the present group of claims.

At paragraph 1 of the Office Action the Examiner maintained the restriction requirement and made it final. Applicants again respectfully request that the restriction requirement with respect to Groups A – F be withdrawn. Applicants' invention concerns methods of increasing production of cysteine, glutathione, methionine or sulfur-containing derivatives of methionine by plant cells and plants which method is accomplished by overexpressing SAT in plant cells transformed with a SAT or in plants containing such transformed cells. The specification at pages 9 and 10 discloses that the overexpressed SAT can be any SAT, whether of plant, bacterial or other origin and provides examples of SATs suitable for use in the invention, including the SAT's in Groups A – F, bacterial SAT, SAT3, SAT3', SAT1, SAT2, and SAT4, respectively. The SATs of Groups A – F are different forms of the same enzyme. Group A is bacterial SAT, whereas Groups B – F are isoforms of plant SAT. As disclosed in the specification, and as known in the art, serine acetyltransferase exists in three forms in plants, cytoplasmic, mitochondrial and chloroplast. SAT3 of Group B is a cytosolic form of SAT. SAT3' of Group C is another cytosolic form of SAT from *Arabidopsis thaliana*. SAT1 of Group D is a mitochondrial form of SAT. SAT 2 of Group E is a chloroplast form of SAT. SAT4 of Group F is also a chloroplast form of SAT. Each of the SATs has

the same function and effect, and is used in the same manner in the claimed methods. The SATs of Groups A – F thus have corresponding technical features and relate to a single general inventive concept under PCT Rule 13.1 and should be examined together in the same application.

At paragraph 2 of the Office Action, the Examiner objected to the drawings. Marked up copies of Figures 1-12 with changes shown in red are submitted for the Examiner's approval in a separate paper.

At paragraph 3 of the Office Action, the Examiner required a substitute specification because of dark smudges on the pages. The Examiner also pointed out that the specification does not comply with 37 CFR 1.821(d) because the sequence identifiers do not correspond with those in the Sequence Listing.

Applicants' attorney Liza Hohenschutz spoke with Examiner Kubelik by telephone on March 4, 2002 regarding the requirement for a substitute specification. Examiner Kubelik advised Applicants' attorney that a clean photocopy of the specification as filed would be acceptable instead of a substitute specification. Applicants accordingly submit herewith a photocopy of the specification, claims and Sequence Listing as filed.

The specification has been amended to insert references to sequence identifiers corresponding to those in the Sequence Listing.

At paragraph 4 of the Office Action, the Examiner objected to the specification because it contains embedded hyperlinks and/or other forms of browser-executable code and deletion of such material.

A review of the specification indicates that page 41, line 15 contains the internet web address of a program that was used in comparing nucleotide sequences. This reference to a program is not an embedded hyperlink or other form of browser-executable code and is not required to be deleted from the specification. Reference to this program has not been deleted from the specification.

At paragraph 5 of the Office Action, the Examiner objected to the Abstract. A new Abstract is submitted herewith.

At paragraphs 6 and 7 of the Office Action, the Examiner rejected claims 1-6, 8, 17-20 and 23-30 under 35 USC 112, first paragraph as not enabled. The basis for this rejection is that the specification does not provide enablement for methods of increasing production of cysteine, glutathione, methionine and sulfur derivatives in a plant by overexpressing SAT from any plant, by overexpressing a cysteine-sensitive SAT, by overexpressing SAT by mitochondrial transformation, for mutagenesis of a plant SAT, for expressing in the cytoplasm of an SAT encoded by the chloroplast, or for chloroplast targeting via use of an optimized transit peptide.

Applicants traverse this rejection. The specification provides ample guidance for one skilled in the art to practice the aspects of the invention pointed out as not enabled.

Prior to filing the present application, SATs had been well characterized in bacteria and plants, and a number of references to cloned SATs are cited in the specification at pages 6 and 9. Suitable SAT genes are also disclosed in the figures of the application. Methods for cloning SAT genes are known in the art and are additionally described in the specification in the examples.

Examples of cysteine-sensitive SATs are disclosed in the specification at page 9, lines 14-21. The use of a cysteine-sensitive SAT, SAT1 to increase production of cysteine and methionine in transgenic plants is shown in Example 13.

The specification at page 10 describes the overexpression of a chloroplast SAT in the cytoplasm. For overexpression in the cytoplasm, as disclosed in the specification, the chloroplast signal peptide is removed. This will result in the expression of SAT in the cytoplasm where it will remain since the signal peptide necessary to transport it to the chloroplasts is missing. Example 9 at page 44 discloses a vector, shown in Figure 12, suitable for expression of any SAT in the cytoplasm of a plant cell, including a chloroplast SAT. Persons skilled in the art can readily overexpress chloroplast SAT in the cytoplasm using the guidance of the specification.

With regard to claim 12, overexpression of SAT in the mitochondria, as disclosed in the specification at page 11, lines 1-14 can be accomplished by expression of a mitochondrial signal peptide/SAT fusion protein in the cytoplasm with translocation of the SAT to the mitochondria. Persons skilled in the art can therefore readily overexpress SAT in mitochondria using the guidance of the specification.

The specification discloses at page 9, that mutant forms of SAT can be used in the methods of the invention. Plant and bacterial SATs that have been rendered cysteine-insensitive by mutagenesis are referred to in the specification at page 9, lines 22-27 and the references cited therein.

With regard to the Examiner's remarks about claim 18 and the lack of direction in the specification for methods of transformation of the chloroplast that would direct the protein out of the chloroplast and into the cytoplasm (which the Examiner states is not

currently possible), it appears the dependency of claim 17, upon which claim 18 depends, was incorrect. Claim 17 has been amended to depend from new claim 60 which replaces canceled claim 1.

Chloroplast targeting via an optimized transit peptide (OTP) is disclosed at page 14, lines 16-28 and page 15, lines 1-5. The specification specifically discloses the optimized transit peptide of European patent application EP 508 909, wherein the sunflower RuBisCO ssu transit peptide is fused to a peptide made of the 22 N-terminal amino acids of the mature maize RuBisCO ssu, which is in turn fused to the to the maize RuBisCO ssu transit peptide. This is the OTP shown the plasmid in Figure 11 and referred to in Example 9 at page 43 of the specification.

The specification enables persons skilled in the art to practice the aspects of the invention the Examiner asserted are not enabled. Withdrawal of this section 112, first paragraph rejection is respectfully requested.

At paragraph 8 of the Office Action, the Examiner rejected claims 1, 6, 17, 19, 23, 25, and 27-30 under 35 USC 112, first paragraph because use of a plasmid containing an optimized transit peptide is not enabled and hence the invention is not enabled.

Applicants traverse this rejection. As discussed above, an optimized transit peptide (OTP) is disclosed in the specification at page 14, lines 16-28 and page 15, lines 1-5. The specification specifically discloses the optimized transit peptide of European patent application EP 508 909, wherein the sunflower RuBisCO ssu transit peptide is fused to a peptide made of the twenty-two N-terminal amino acids of the mature maize RuBisCO ssu, which is in turn fused to the to the maize RuBisCO ssu transit peptide.

This is the OTP shown the plasmid in Figure 11 and referred to in Example 9 at page 43 of the specification. This OTP is readily available to persons skilled in the art.

An OTP is not essential for practicing the present invention. Other chloroplast transit peptides are known in the art and are suitable for use with the present invention. The specification at page 13 discloses chloroplast transit peptides. Additional transit peptides can be obtained from the sequences of SAT genes disclosed herein that contain transit peptides.

The specification enables an optimized transit peptide and also enables other chloroplast transit peptides suitable for use in overexpressing SAT in chloroplasts. Withdrawal of this section 112, first paragraph rejection is requested.

A paragraph 9 of the Office Action, the Examiner rejected claims 1-6, 8, 12-13, 17-20 and 23-30 under 35 USC 112, first paragraph because the specification fails to provide a written description of SATs from any species other than *Arabidopsis* or for any mutant SATs or any optimized transit proteins (OTP).

Applicants traverse this rejection. Applicants have provided sufficient written description of the invention for persons skilled in the art to practice the claimed invention. SAT is a well-characterized enzyme. The specification at pages 9 and 10 discloses that the overexpressed SAT can be any SAT, whether of plant, bacterial or other origin, and provides examples of SATs suitable for use in the invention, including cysteine-sensitive and cysteine-insensitive forms of SAT, and cytoplasmic, mitochondrial and chloroplast forms of SAT. In addition to SAT3 (SEQ ID NO: 1), the application discloses the sequences of five other SATs. Persons skilled in the art can readily substitute any other SAT for the ones disclosed in the specification by referring to the

scientific literature and databases. For example, Saito *et al.*, Gene 189: 57-63, 1997, cited in the present Office Action, discloses the nucleotide and amino acid sequence of a cytoplasmic SAT from watermelon.

Plant and bacterial SATs that have been rendered cysteine-insensitive by mutagenesis are referred to in the specification at page 9, lines 22-27 and the references cited therein.

As discussed above, an optimized transit peptide (OTP) is disclosed in the specification at page 14, lines 16-28 and page 15, lines 1-5. The specification specifically discloses the optimized transit peptide of European patent application EP 508 909, wherein the sunflower RuBisCO ssu transit peptide is fused to a peptide made of the twenty-two N-terminal amino acids of the mature maize RuBisCO ssu, which is in turn fused to the to the maize RuBisCO ssu transit peptide.

The specification provides sufficient written description for one skilled in the art to practice the claimed invention. Withdrawal of this section 112, first paragraph rejection is requested.

At paragraph 10 of the Office Action, the Examiner rejected claims 1-6, 8-9, 12, 13, 17-20 and 23-30 under 35 USC 112, second paragraph, as being indefinite. This rejection was lengthy (three and one half pages long). Each specific rejection will not be repeated here.

Claims 2-6, 9, 12, 13, 17-20 and 23-25 have been amended. Claims 1, 8, 14, and 27-30 have been canceled without prejudice and replaced with new claims 60-64 and 66-71. The claim amendments and new claims incorporate responses to each point of the instant rejection.

The abbreviation SAT has been removed from the claims and replaced with serine acetyltransferase. Claim 26 has not been amended to remove the abbreviation "EPSPS". Applicants believe this abbreviation is well-known in the art and is not indefinite.

The terms "homologous" and "heterologous" in claims 20 and 21 are also not indefinite. The specification at page 13, lines 9-20 indicates that "homologous" refers to the situation where the transit peptide and SAT are the ones naturally expressed in chloroplasts (or mitochondria). "Heterologous" refers to the situation where the transit peptide is not naturally associated with the SAT, such as where a chloroplast SAT transit peptide is linked with a cytosolic SAT, or a transit peptide from a chloroplast protein other than a chloroplast SAT is linked with SAT.

The term "sulphur derivatives thereof" in claim 1, now rewritten as new claim 60, has been amended to "sulfur-containing derivatives of methionine". Support for this amendment can be found in the specification at page 8, line 17. As shown in Figure 2, methionine is the precursor for S-methylmethionine and S-adenosylmethionine.

Withdrawal of this section 112, second paragraph rejection is requested.

At paragraph 12 of the Office Action, the Examiner rejected claims 1-6, 8-9, 12, 13, 17-20 and 23-30 under 35 USC 112, second paragraph as being incomplete for omitting essential steps. The Examiner indicated that method steps must be circular and the final step must generate the item the method is intended to produce.

Applicants traverse this rejection. Claim 1, now rewritten as new claim 60, does not omit essential steps. Claim 60 states that the plant cells are transformed with a nucleotide sequence encoding an SAT, and also states that overexpression of SAT in the plant cells or plants containing such cells results in the increased production of cysteine,

methionine, glutathione, methionine or sulfur-containing derivatives of methionine. Additional steps are not required to produce the claimed increased production of cysteine, methionine, glutathione, methionine or sulfur-containing derivatives of methionine. Withdrawal of this section 112, second paragraph rejection is requested.

At paragraphs 13 and 14 of the Office Action, the Examiner rejected claims 1, 4-6, 8, 12, 13, and 24 under 35 USC 102(b) as being anticipated by Takahashi *et al.*, PNAS 94: 11102-11107, 1997. The Examiner stated that Takahashi *et al.* teaches SAT1 is overexpressed in *Arabidopsis* upon sulfur starvation. The Examiner further stated that SAT1 encodes an insensitive mitochondrial form of SAT and thus is expressed in the cytoplasm before transport to the mitochondria; after transport, it would be expressed there.

Applicants traverse this rejection. Takahashi *et al.* discloses isolation and characterization of a sulfate transporter gene from *Arabidopsis thaliana*, AST68. Takahashi *et al.* also discloses experiments on the effects of sulfite starvation of *A. thaliana* plants. The *A. thaliana* plants used in experiments were not transgenic plants. Three genes AT68 (the sulfate transporter gene), APR1 and SAT1 were induced by sulfate starvation. SAT1 levels increased 3.5-fold in leaves and 2-fold in roots. SAT-A and SAT53 (Accession no. U30298), the two other isoforms of SAT did not increase in response to sulfate starvation.

Takahashi *et al.* does not anticipate 1 (now rewritten as new claim 60), or claims 4-6, 12, 13, 24 and 8 (now rewritten as new claims 61 and 62) which depend, directly or indirectly from claim 60. Any increase in expression of SAT in Takahashi *et al.* is the result of stress upon native untransformed plants. By contrast the methods of claims 4-

6,12, 13, 24, 60, 61 and 62 require plant cells transformed with a nucleotide sequence encoding SAT. Such transformed cells are not disclosed in Takahashi *et al.*

Additionally, Takahashi *et al.* does not disclose a method of increasing the production of cysteine by overexpressing SAT in plant cells transformed with a nucleotide sequence encoding SAT. Withdrawal of this section 102(b) rejection is requested.

At paragraph 15 of the Office Action the Examiner rejected claims 1-3, 6, 8, and 24 under 35 USC102(b) as being anticipated by Saito *et al.*, Gene 189: 57-63, 1997 in light of Noji *et al.*, JBC 273:32739-32745, 1998. The basis for this rejection is that Saito *et al.* teach SAT is overexpressed in watermelon upon sulfur starvation and by addition of pyrazole, and Noji *et al.* teach that this SAT is a cystolic form of SAT and is sensitive to cysteine.

Saito *et al.* discloses isolation and characterization of a genomic nucleic acid sequence encoding SAT from watermelon. This publication also discloses experiments on the expression pattern of SAT under sulfur- and nitrogen-starved conditions, and experiments on induction of SAT mRNA accumulation. All of these experiments were done with untransformed watermelon plants. Noji *et al.* discloses experiments on the subcellular localization of SAT and feed back regulation of the three SAT forms from *A. thaliana*. Noji *et al.* also discloses that the watermelon SAT is present in the cytosol and is sensitive to cysteine. Experiments were performed using *E. coli* or plants transformed with fusion proteins comprised of an SAT N-terminal fragment fused with jellyfish green fluorescent protein. Claim 1, now rewritten as new claim 60, is drawn to a method that requires plant cells transformed with a nucleotide sequence encoding SAT and plants

containing such cells. Claims 2, 3, 6, 24 and 8 (new rewritten as new claims 61 and 62) which depend, directly or indirectly, from claim 60 also incorporate this limitation. Neither Saito *et al.* nor Noji *et al.* teach a method of increasing the production of cysteine, glutathione, methionine or sulfur-containing derivatives of methionine in plant cells and plants by overexpressing SAT. Saito *et al.* in light of Noji *et al.* does not anticipate claims 2, 3, 6, 24, 60, 61 and 62. Withdrawal of this section 102(b) reference is requested.

At paragraphs 16 and 17 the Examiner rejected claims 1-6, 8, 12-13, 17, 19, 20 and 23-30 under 35 USC 103(a) as being unpatentable over Saito *et al.*, Plant Physiol. 106: 887-895, 1994 in view of Noji *et al.* (supra). The basis for this rejection is that it would have been obvious to one of ordinary skill in the art to increase the production of cysteine in a plant by overexpressing a cytoplasmic cysteine synthase in the cytoplasm and chloroplasts of a plant as taught by Saito *et al.* and to modify that to use another enzyme required for cysteine biosynthesis, SAT, as described in Noji *et al.* and that one skilled in the art would be motivated to do so because of the role SAT has in regulation of cysteine biosynthesis and because substitution of one crucial enzyme for cysteine biosynthesis for another crucial enzyme for cysteine biosynthesis is an obvious design choice.

Applicants traverse this rejection. Saito *et al.* discloses overexpression of cysteine synthase in tobacco plants transformed to express spinach cytoplasmic cysteine synthase. Plants were transformed with vectors containing cysteine synthase alone in the sense and antisense directions, or fused with a chloroplast transit peptide. Cysteine synthase is responsible for the terminal step of cysteine biosynthesis and catalyzes the

formation of L-cysteine from O-acetylserine and hydrogen sulfide. Saito *et al.* found that cell-free extracts of plants transformed with cysteine synthase had 2- to 3- fold higher activity than those of control plants and plants where the cysteine synthase was inserted in the antisense direction. Isolated chloroplasts of a plants transformed with the cysteine synthase fused with a chloroplast transit peptide had a more pronounced ability to form cysteine in response to addition of O-acetylserine and sulfur compounds than those of a control plant. Conversely, there were no significant changes in the cellular content of cysteine and glutathione in the transgenic plants, although there was some tendency toward an increase in comparison to control plants. Saito *et al.* concluded that this suggested that under normal growth conditions without sulfur stress, the cellular contents of cysteine and glutathione were stationary and not directly influenced by increased cellular cysteine synthase activity. (Saito *et al.*, page 890, last paragraph to page 891, first paragraph). The experiments in Saito *et al.* using isolated chloroplasts merely shift the equilibrium of the reaction leading to the synthesis of cysteine by adding the substrates for cysteine synthase, O-acetylserine and sulfite to isolated chloroplasts. Saito *et al.* thus do not teach a method of increasing the production of cysteine in plants. Additionally, there is also no suggestion of the importance of SAT in the production of cysteine.

As discussed above, Noji *et al.* discloses experiments on the subcellular localization of SAT and feed back regulation of the three SAT forms from *A. thaliana*. Experiments were performed using *E. coli* or plants transformed with fusion proteins comprised of an SAT N-terminal fragment fused to jellyfish green fluorescent protein (GFP). Noji *et al.* discusses the putative role of SAT in regulation of cysteine

biosynthesis by analogy to bacterial SAT. There is no suggestion in Noji *et al.* that overexpression of SAT in plant cells would increase production of cysteine.

It is well-established that before a conclusion of obviousness may be made based on a combination of references, there must be a reason, suggestion or motivation in the prior art to lead an inventor to combine those references. Saito *et al.* has nothing in common with the present invention. Saito *et al.* discloses experiments using a different enzyme, cysteine synthase. Saito *et al.* does not disclose a method of increasing cysteine production in plants, much less suggest that SAT could be substituted for cysteine synthase. Noji *et al.* does not cure the deficiencies of Saito *et al.* Noji *et al.* discloses experiments of feedback regulation and subcellular localization of SAT, but does not suggest that overexpression of SAT in plant cells could increase production of cysteine.

Even assuming *arguendo* that the combination of references were proper, nevertheless, the combined disclosures of the references are insufficient to support the Examiner's conclusion that the claimed methods of increasing the production of cysteine is obvious. The combined disclosures of Saito *et al.* and Noji *et al.* still fail to suggest that cysteine production could be increased by overexpressing SAT in plant cells. Saito *et al.* does not disclose a method for increasing production of cysteine in plant cells. Saito *et al.* overexpressed a different enzyme, cysteine synthase, in tobacco plants, but this did not result in a significant increase in levels of cysteine and the authors concluded that under normal conditions, the cellular contents of cysteine and glutathione were stationary and not directly influenced by increased cellular cysteine synthase activity. Noji *et al.* discusses the putative role of plant SAT in regulation of cysteine biosynthesis

by analogy to bacterial SAT, but does not suggest overexpression of SAT in plants to increase cysteine production.

The Examiner's reasons for combining Saito *et al.* and Noji *et al.* amount to an impermissible hindsight reconstruction of Applicant's invention and do not stem from the prior art. Synthesis of cysteine in plants depends on a number of enzymes and requires the availability of biosynthetic precursors. In addition, sulfate transporters play an important role. Given this complex regulation of sulfur assimilation, the present invention surprisingly shows that the overexpression of a single enzyme, SAT, allows increased production of sulfur-containing amino acids in plants.

Claims 1 (now rewritten as new claim 60), 2-6, 8 (new rewritten as new claims 61 and 62_ 12, 13, 17, 19, 20, 23-26 and 27-30 (new rewritten as new claims 66-71) are not obvious over Saito *et al.* in view of Noji *et al.*. Withdrawal of this section 103(a) rejection is requested.

At paragraph 18 of the Office Action, the Examiner rejected claim 9 under 35 USC as being unpatentable over Saito *et al.* in view of Noji *et al.* as applied in the previous rejection, and further in view of Ruffett *et al.*, Eur. J. Biochem 227: 500-509. The basis for this rejection is the same as the previous section 103(a) rejection, with the additional rationale that one skilled in the art would be motivated to modify the teachings to of these references to use another SAT isoform as described in Ruffett *et al.* because substitution of *Arabidopsis* SAT genes would be an obvious design choice and because the SAT taught by Ruffett *et al.* is apparently cysteine-insensitive.

Applicants traverse this rejection. Saito *et al.* and Noji *et al.* have been discussed above. Ruffett *et al.* discloses experiments on the subcellular distribution of SAT from

Pisum sativum and isolation and characterization of a cytosolic isoform of SAT from *A. thaliana* referred to as SAT5. There is no suggestion in Ruffett *et al.* that cysteine production in plants could be increased by overexpressing SAT.

The addition of Ruffett *et al.* does not cure the deficiencies of Saito *et al.* and Noji *et al.* The combined disclosures of Saito *et al.*, Noji *et al.* and Ruffett *et al.* still fail to disclose increasing the production of cysteine in plants by overexpressing SAT or any suggestion that doing so would increase production of cysteine.

Claim 9 is not obvious over Saito *et al.* in view of Noji *et al.* and Ruffett *et al.* Withdrawal of this section 103(a) rejection is requested.

At paragraph 19 of the Office Action, the Examiner rejected claim 18 under 35 USC 103(a) as being unpatentable over Saito *et al.* in view of Noji *et al.* and further in view of Svab *et al.*, PNAS 90: 913-917, 1993. The basis for this rejection is the same for the section 103(a) rejection at paragraphs 16 and 17, with the additional rationale that it would have been an obvious design choice to substitute nuclear transformation with a construct that has a chloroplast transit peptide with introduction of the protein into the chloroplast by chloroplast transformation.

Applicants traverse this rejection. Saito *et al.* and Noji *et al.* have been discussed above. Svab *et al.* discloses increased frequency of plastid transformation using a plasmid containing a tobacco *SacII-EcoRV* plastid fragment wherein a chimeric gene is inserted between the *rbcL* gene and open reading frame ORF512. Svab *et al.* has nothing in common with the present invention beyond the disclosure of a method for transforming chloroplasts that could be used transform chloroplasts with SAT.

The addition of Svab *et al.* does not cure the deficiencies of Saito *et al.* and Noji *et al.* The combined disclosures of Saito *et al.*, Noji *et al.* and Svab *et al.* still fail to disclose increasing the production of cysteine in plants by overexpressing SAT or any suggestion that doing so would increase production of cysteine.

Claim 18 is not obvious over Saito *et al.* in view of Noji *et al.* and Svab *et al.* Withdrawal of this section 103(a) rejection is requested.

In view of the above, the present application is believed to be in a condition ready for allowance. Reconsideration of the application is respectfully requested and an early Notice of Allowance is earnestly solicited.

Respectfully submitted,

CONNOLLY BOVE LODGE & HUTZ LLP

Date: March 11, 2002

By: Liza D. Hohenschutz
Liza D. Hohenschutz
Reg. No. 33,712
P.O. Box 2207
Wilmington, Delaware 19899
Attorney for Applicants

**APPENDIX A****Marked Up Version Of Amended Specification**

Paragraph at page 10, lines 5 – 24:

According to a first embodiment of the invention, the SAT is overexpressed in the cytoplasm of the plant cells. The SAT is either a plan cytoplasmic SAT, in particular the SAT3 (L34076) or SAT3' or SAT52 (U30298), represented by [the SEQ IN NO 1] SEQ ID NO: 1 or [the SEQ ID NO 2] SEQ ID NO: 3, respectively, or an SAT of bacterial origin as defined above. The SAT overexpressed in the cytoplasm can also be a noncytoplasmic plant SAT, for example a chloroplast or mitochondrial SAT. These noncytoplasmic plant SATs, naturally, are expressed in the cytoplasm in the form of a precursor protein comprising a signal for addressing to the cellular compartment, other than the cytoplasm, into which the mature functional SAT is released. In order to overexpress these mature functional SATs in the cytoplasm, their addressing signal is removed. In this case, the SAT protein overexpressed in the cytoplasm is a noncytoplasmic plant SAT, with its signal(s) for addressing to cellular compartments, other than the cytoplasm, removed.

Paragraph at page 10, lines 25-27.

According to a specific embodiment of the invention, the noncytoplasmic SAT with its addressing signal removed is SAT1' represented by [SEQ ID NO 3] SEQ ID NO: 5.

Paragraph at page 11, lines 1 – 13:

According to a second embodiment of the invention, the SAT is overexpressed in the mitochondria. The protein is advantageously expressed in the cytoplasm in the form of a signal peptide/SAT fusion protein, the mature functional SAT being released inside the mitochondria. Advantageously, the mitochondrial addressing signal peptide is made up of at least one mitochondrial addressing signal peptide from a plant protein which is located in mitochondria, such as the tobacco ATPase β -F1 subunit signal peptide [[25] Hemon P. *et al.* 1990, Plant Mol. Biol. 15, 895-904], or the SAT1 signal peptide represented by amino acids 1 to 63 in [SEQ ID NO 4] SEQ ID NO: 8.

Paragraph at page 11, lines 14-16:

According to a specific embodiment of the invention, the mitochondrial SAT is SAT1 (U22964) represented by [SEQ ID NO 4] SEQ ID NO: 8.

Paragraph at page 12, lines 17 – 19:

In this case, the SAT can be a chloroplast SAT of plant origin, such as SAT2 or SAT4, represented by [SEQ ID NO 5 or 6] SEQ ID NO: 9 and 11, respectively.

Paragraph at page 13, lines 9 – 24

In the fusion protein according to the invention, the SAT can be homologous or heterologous to the transit peptide. In the first case, the fusion protein is the SAT2 or the SAT4 protein expressed naturally in the chloroplasts of plant cells. In the second case, the transit peptide can be a transit peptide from an SAT2, represented by amino acids 1 to 32 of [SEQ ID NO 5] SEQ ID NO: 9, or the transit peptide from an SAT4, represented

by amino acids 1 to 30 of [SEQ ID NO 6] SEQ ID NO: 11, or alternatively a transit peptide from another protein, which is located in plastids, in particular the transit peptides defined below. Plastid localization protein is understood to mean a protein expressed in the cytoplasm of plant cells in the form of a transit peptide/protein fusion protein, the mature protein being localized in the chloroplast after cleavage of the transit peptide.

Paragraph at page 34, lines 3 – 10:

A gene encoding a putative cytosolic serine acetyltransferase (Z34888 or L34076) represented in **Figure 4** [(SEQ ID NO 1)] (SEQ ID NO: 1), was isolated by functional complementation of an *Escherichia coli* strain deficient in serine acetyltransferase activity. Analysis of the primary sequence showed the presence of strong similarity with the sequence of the bacterial enzyme (56% homology and 41% identity).

Paragraph at page 34, lines 11 – 14:

The following primers were used to amplify the nucleotide sequence and to clone it into the vector used for transforming tobacco plants:

Oligo 1: 5'GAGAGAGGAT CCTCTTTCCA ATCATAAACC ATGGCAACAT
GCATAGACAC ATGC 3' (SEQ ID NO: 13)

Oligo 2: 5'GGCTCACCAG ACTAATACAC TAAATTGTGT TTACCTCGAG
AGAGAG 3' (SEQ ID NO: 14)

Paragraph at page 36 lines 14-16:

The procedure of Example 3 is repeated, using oligonucleotides 3 and 4 below:

Oligo 3: 5'GAGAGAGGAT CCTCTTATCG CCGCGTTAAT ATGCCACCGG
CCGGAGAACTC C 3' (SEQ ID NO: 15)

Oligo 4: 5'GAGCCTTACC AGTCTAATGT AGTATATTTC AACCTCGAGA
GAGAG 3' (SEQ ID NO: 16)

Paragraph at page 36, line 17 through page 37, line 8:

A gene is isolated which encodes an acetyltransferase (U 30298), and is represented in **Figure 5** [(SEQ ID NO 2)] (SEQ ID NO: 3). Analysis of the primary sequence showed the presence of strong similarity with the sequence of the bacterial enzyme (51.6% homology and 42.6% identity). The N-terminal structure (absence of the conditions necessary for organelle addressing) indicates that this isoform is located in the cytosol. On the other hand, it is given as being cysteine-sensitive [27]. This result differs from the data obtained from pea leaves (and from spinach leaves), in the sense that the cysteine regulation site seems to be confined to the cytosol in *A. thaliana* [27].

Moreover, it would seem that *A. thaliana* has at least two cytosolic isoforms: SAT3 (Example 3) and SAT3' (or U30298, Example 4). Unlike the SAT3 gene, the gene corresponding to SAT3' has an intron.

Paragraph at page 37, line 14-21:

A gene encoding a serine acetyltransferase (L78443), which is represented in **Figure 6** [SEQ ID NO 3)] (SEQ ID NO: 5), was isolated by functional complementation of an *Escherichia coli* strain deficient in serine acetyltransferase activity [12]. Analysis

of the primary sequence shows strong similarity with the sequence of the bacterial enzyme (52.7% homology and 39% identity).

Paragraph at page 37, line 22 through page 38, line1:

The following primers were used to amplify the nucleotide sequence and to clone it into the vector which is used for transforming tobacco plants (in bold characters in **Figure 3**):

Oligo 5: 5'GAGAGAGGAT CCCCTCCTCC TCCTCCTCCT ATGGCTGCGT
GCATCGACAC CTG 3' (SEQ ID NO: 17)

Oligo 6: 5'GCTCACCAGC CTAATACATT AAAC TTTTTC AGCTCGAGAG
AGAG 3' (SEQ ID NO: 18)

Paragraph at page 38, lines 5 – 10:

A second gene is obtained which encodes a putative mitochondrial serine acetyltransferase (U22964), and is represented in **Figure 7** [(SEQ ID NO 4)] (SEQ ID NO: 7), by repeating the same procedure, using oligo 7 to replace oligo 5 as the 5' primer.

Oligo 7: 5'GAGAGAGGAT CCGGCCGAGA AAAAAAAAAA ATGTTGCCGG
TCACAAGTCG CCG 3' (SEQ ID NO: 19)

Paragraph at page 39, lines 27 through page 40, line 8, and insert the following.

A mitochondrial fraction lacking in plastid and in cytosolic contaminants was obtained by using the protocol defined for pea leaf mitochondria [12]. The molecular

mass of the polypeptide as revealed by antibodies raised against the peptide [-TKTLHTRPILLEDLDLDR-] (SEQ ID NO: 5, amino acids 49-63) (see SAT1 amino acid sequence), is of the order of 34,000 daltons, a value which is in agreement with the mass of the protein as obtained using sequence analysis programs for predicting cleavage sites.

Paragraph at page 40, lines 14 – 21:

A gene which encodes a serine acetyltransferase (L78444), represented in **Figure 8** [(SEQ ID NO 5)] (SEQ ID NO: 9), was isolated by functional complementation of an *Escherichia coli* strain deficient in serine acetyltransferase activity [12]. Analysis of the primary sequence showed the presence of strong similarity with the sequence of the bacterial enzyme (49.5% homology and 35.4% identity).

Paragraph at page 40, line 22 through page 21, line 1:

The following primers were used to amplify the nucleotide sequence and to clone it into the vector which was used to transform tobacco plants (in bold characters in **Figure 8**):

Oligo 8: 5'GAGAGAGGAT CCGACAAGTT GGCATAATTT ATGGTGGATC
TATCTTCCT 3' (SEQ ID NO: 20)

Oligo 9: 5'CCTGTGTGAT TGTCGTGTAG TACTCTAGAA ACTCGAGAGA
GAG 3' (SEQ ID NO: 21)

Paragraph at page 41, line 21 through page 42, line 3:

A gene which encodes a serine acetyltransferase (SAT4), represented in **Figure 9** [(SEQ ID NO 6)] (SEQ ID NO: 11), was isolated by functional complementation of an *Escherichia coli* strain deficient in serine acetyltransferase activity [12]. Analysis of the primary sequence showed the presence of strong similarity with the sequence of the bacterial enzyme (44.5% homology and 32% identity).

Paragraph at page 42, lines 4 – 8:

The following primers were used to amplify the nucleotide sequence and to clone it into the vector which was used for transforming tobacco plants:

Oligo 10: 5'GAGAGAGGAT CCGACAAGTTGG CATAATTTAT
GGCTTGATA AACGGCGAGA ATCGTGATTT TTCTT
(SEQ ID NO: 22)

Oligo 11: 5'TACCTCGTAC CACTCAGAAC TCTAGAAACT
CGAGAGAGAG3' (SEQ ID NO: 23)

Paragraph at page 43, lines 17 – 21:

To obtain expression of the SAT3 [(SEQ ID NO 1)] (SEQ ID NO: 1) of Example 2 in the chloroplast (**Figure 11**), an extension which allows addressing to this compartment is introduced 5' of the cDNA. For this, the optimized transit peptide previously described is used.

APPENDIX B**Marked Up Version Of Amended Claims.**

2. (amended) Method according to claim [1] 60, characterized in that the [SAT] serine acetyltransferase which is overexpressed in plant cells is a cysteine-sensitive [SAT] serine acetyltransferase.
3. (amended) Method according to claim 2, characterized in that the [SAT] serine acetyltransferase is a plant [SAT] serine acetyltransferase or a [native SAT of bacterial origin] bacterial serine acetyltransferase.
4. (amended) Method according to claim [1] 60, characterized in that the [SAT] serine acetyltransferase which is overexpressed in plant cells is a cysteine-insensitive [SAT] serine acetyltransferase.
5. (amended) Method according to claim 4, characterized in that the [SAT] serine acetyltransferase is a plant SAT, [or an SAT of bacterial origin] a bacterial serine acetyltransferase, [or] a [mutated] plant SAT [,] rendered cysteine-insensitive by mutagenesis or a bacterial serine acetyltransferase rendered cysteine-insensitive by mutagenesis.
6. (amended) Method according to claim [1] 60 characterized in that the [SAT] serine acetyltransferase is overexpressed in the cytoplasm of plant cells.
9. (amended) Method according to claim [8] 62, characterized in that the [SAT] serine acetyltransferase is SAT3 which is represented by [SEQ ID NO 1] SEQ ID NO: 2.
12. (amended) Method according to claim [1] 60, characterized in that the [SAT] serine acetyltransferase is overexpressed in mitochondria.

13. (amended) Method according to claim 12, characterized in that the [SAT] serine acetyltransferase is overexpressed in the cytoplasm in the form of a mitochondrial signal peptide/[SAT] serine acetyltransferase fusion protein, the mature functional [SAT] serine acetyltransferase being released inside mitochondria.

17. (amended) Method according to claim [6] 60, characterized in that the [SAT] serine acetyltransferase is overexpressed in chloroplasts of plant cells.

18. (amended) Method according to claim 17, characterized in that the [SAT] serine acetyltransferase is overexpressed in chloroplasts by integration, into chloroplast DNA of plant cells, of a chimeric gene comprising a DNA sequence encoding the [said SAT] serine acetyltransferase, under the control of 5' and 3' regulatory elements which are functional in chloroplasts.

19. (amended) Method according to claim 17, characterized in that the [SAT] serine acetyltransferase is overexpressed in the cytoplasm in the form of a transit peptide/[SAT] serine acetyltransferase fusion protein, the mature functional [SAT] serine acetyltransferase being released inside chloroplasts.

20. (amended) Method according to claim 19, characterized in that the [SAT is homologous with the transit peptide] serine acetyltransferase and transit peptide of the fusion protein are homologous.

23. (amended) Method according to claim 19, characterized in that the [SAT is heterologous with the transit peptide] serine acetyltransferase and the transit peptide of the fusion protein are heterologous.

24. (amended) Method according to claim 13, characterized in that the [SAT] serine acetyltransferase is a plant cytoplasmic [SAT of plant origin] serine

acetyltransferase or [an SAT of bacterial origin] a bacterial serine acetyltransferase [, and that the SAT is a plant SAT or a native SAT of bacterial origin].

25. (amended) Method according to claim 23, characterized in that the transit peptide is a transit peptide from [another protein which is located in plastids] a plastid protein other than a chloroplast serine acetyltransferase.